Accession No. 408937-05 and
411114-01 (Supplement)

DATA EVALUATION RECORD

1. **CHEMICAL:** Sulfosate Technical.
Shaughnessey No. 128501.

2. **TEST MATERIAL:** 1) SC-0224 Technical, Lot No. WRC 10387-47-01, Sample purity: 57.3%, a light amber liquid; 2) ^{14}C-SC-0224, Lot No. WRC-8917-23-01, Radiochemical purity: 96.4%, an amber liquid.

3. **STUDY TYPE:** Life Cycle Test with Freshwater Invertebrate.
Species Tested: *Daphnia magna*.

4. **CITATION:** Forbis, A.D. 1987. Chronic Toxicity of ^{14}C-SC-0224 to *Daphnia magna* Under Flow-Through Test Conditions. Prepared by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. ABC Report No. 35820. Submitted by ICI Americas Inc., Farmington, Connecticut. Accession No. 408937-05 and 411114-01 (Supplement).

5. **REVIEWED BY:**

Kimberly D. Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Kimberly D. Rhodes
Date: July 10, 1989

6. **APPROVED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: July 11, 1989

Henry T. Craven
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: Sept 6/89

CLYDE R. HOUSEKNECHT, BIOLOGIST — Clyde R. Houseknecht 9/20/89

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater invertebrate life cycle test. The MATC of ^{14}C-SC-0224 for *Daphnia magna* was > 1.2 < 2.1 mg/L based on significant reductions in length and young produced/adult/reproduction day. A 21-day EC50 was calculated to be 2.7 mg/L.

8. **RECOMMENDATIONS:** N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: The daphnids (Daphnia magna) used to initiate the life cycle test exposure were obtained from the culture unit at the testing facility. All daphnids were cultured and tested in a temperature controlled area at 20 ± 2°C. The lighting was 50-70 footcandles on a 16-hour daylight photoperiod, 8-hour darkness and 30-minute transition periods. During the holding period, the daphnids were fed a suspension of algae (Selenastrum capricornutum) supplemented with a Tetramin/cereal leaves/yeast suspension. Only first-instar daphnids (<24 hours old) were selected for testing.

B. Test System: The test was conducted in a half-liter proportional diluter system described by Mount and Brungs (1967), utilizing a Hamilton Micro Lab 420 syringe dispenser. A dilution factor of 50 percent was used. The diluter delivered five concentrations of ^{14}C-SC-0224 and a dilution water control to four replicate one-liter test aquaria. The diluter provided for approximately 6.1 volume replacements per 24-hour period. The diluter stock solution (4.47 mg/L) prepared by adding 1.0 mL of ^{14}C-SC-0224 Primary Stock Solution (4.28 mg/mL) to a 2-L volumetric flask containing 8.94 x 10^3 mg non-radiolabelled SC-0224. The flask was then filled to the mark with de-ionized water.

Illumination was provided by fluorescent lights set on a 16-hour light and 8-hour dark and 30-minute transition photoperiod. Test temperature was maintained at 20 ± 2°C by a temperature controlled water bath. The dilution water was well water characterized as having a total hardness range of 206-275 mg/L as $CaCO_3$, total alkalinity range of 224-336 mg/L as $CaCO_3$, pH range of 7.6-8.4, dissolved oxygen range of 7.4-9.1 mg/L and a conductivity range of 500-650 umhos/cm.

C. Dosage: Twenty-one day flow-through life cycle test.

D. Design: Forty D. magna (< 24 hours old) were impartially distributed to each test concentration (10 per replicate) to initiate the test. A control and nominal ^{14}C-SC-0224 concentrations of 0.27, 0.54, 1.1, 2.2, and 4.5 mg/L were used. Exposure concentrations of ^{14}C-SC-0224 were radiochemically measured on days 0, 4, 7, 14, and 21. The mean measured test concentrations reported were 0.34, 0.65, 1.2, 2.1 and 4.8 mg/L. Water quality parameters of dissolved oxygen and pH were measured on days 0, 4, 7, 14 and 21 in two alternating replicates of the control, low, middle and high test concentrations. Temperature measurements of the water bath were made daily and were also recorded continuously with a data logger.

E. Statistics: The selected parameters of survival, adult length (pooled) and total young/adult/reproduction day were analyzed using a one-way analysis of variance. When treatment effects were indicated following a significant F-test of the mean square ratios, a multiple means comparison test (Dunnett's Test) was used to determine which exposure levels differed from the control value. Percent survival data were transformed for analysis. All differences were considered significant at the 95% confidence level. Total young/adult/reproduction day for each replicate was calculated by dividing the total number of young produced by the total number of adult reproduction days. The number of reproduction days (normally 13-15) were counted from the day instars were first observed, which for this study ranged from Day 9 to Day 12. Adult reproduction days were calculated for each change in survival in order to be corrected for mortality. Total number of adult reproduction days for each replicate was the sum of each adult reproduction day for each change in survival. The 21-day EC50 was calculated by employing a computerized EC50 program developed by Stephan et al.

12. REPORTED RESULTS: A summary of the biological results of the exposure of daphnids to ^{14}C-SC-0224 is provided in the Table 3 (attached). The mean measured concentrations of ^{14}C-SC-0224 during the 21-day exposure ranged from 95 to 126% of expected nominal concentrations. Statistical analysis of survival for Daphnia magna after a 21-day exposure to ^{14}C-SC-0224 indicated that daphnid survival in the ^{14}C-SC-0224 mean measured concentration of 4.8 mg/L was significantly different from the control. The MATC limits

for survival were estimated to be 2.1 and 4.8 mg/L. A 21-day EC_{50} was calculated to be 2.7 mg/L.

The daphnids lengths in ^{14}C-SC-0224 mean measured concentration of 4.8 mg/L were significantly different ($P \leq 0.05$) from the control. The MATC limits for length were estimated to be between 2.1 and 4.8 mg/L. Length measurements could not be made on the 4.8 mg/L test level daphnids since all had died by day four.

The mean young/adult/reproduction day for 21 days were significantly affected in mean measured concentrations of 2.1 and 4.8 mg/L of ^{14}C-SC-0224. The estimated MATC limits for reproduction were 1.2 and 2.1 mg/L. Mean young/adult/reproduction day for 21 days could not be calculated for the 4.8 mg/L test level since all daphnids had died before reaching a reproductive state.

Based on the statistical analysis of survival, mean adult length and young/adult/reproduction day, from this 21-day Daphnia magna dynamic life cycle study, the MATC limits were estimated to be between 1.2 and 2.1 mg/L.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
No conclusions were made by the author.

A GLP compliance statement was included in the report and the study was audited by the QA Unit of ABC Laboratory. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards: Pesticide Programs (40 CFR 160).

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: At the present time, there is no SEP for Daphnia magna flow-through chronic test. Therefore, the ASTM Guidelines were used for this data validation. The report deviated from the ASTM as follows:

o The ASTM states that hardness, alkalinity, and conductivity in the control, low, medium and high concentration test solutions must be measured weekly during the test. This test did not measure these water quality parameters.

B. Statistical Analysis: The reviewer evaluated daphnid survival following an arc-sine square root transformation of the data. Reproduction (young/adult/reproduction day) and growth (length) were statistically evaluated by ANOVA without any transformations. All printouts are attached.

The reviewer confirmed a significant difference (P = 0.01) of survival of daphnids in the highest mean measured test concentration (4.8 mg/L) when compared to the control.

The reviewer also confirmed a significant difference (P = 0.01) of reproduction of daphnids (young/adult/reproduction day) in the two highest mean measured test concentrations (2.1 and 4.8 mg/L) when compared to the control.

The author analyzed the length data using the mean of each replicate instead of individual measurements, thus ignoring the variability among the daphnids within each replicate. However, the analysis performed by the reviewer using individual lengths yielded the same results (i.e., lengths at the two highest treatment concentrations were different from those in the control).

C. Discussion/Results: The study results appear scientifically valid. The maximum acceptable toxicant concentration (MATC) of ^{14}C-SC-0224 Technical for Daphnia magna was estimated to be > 1.2 < 2.1 mg/L mean measured concentration based on reductions in length and young produced/adult/reproduction day.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes. 07/06/89.

5

Shaughnessey No. 128501 Chemical Name Sulfosate (SC-0224 Technical) Chemical Class _____ Page _____ of _____

Study/Species/Lab/ Succession	Chemical % Active	Results				Reviewer/ Date	Valid: Stat
Avian Reproduction,		Group	Dose(ppm)	Effected/Parameters	Mort.(%)	%Chg Inh.	
Species:		Control					
		Treatment I					
Lab:		Treatment II					
		Treatment III					
Acc		Study Duration:					
		Comments:					

Field Study(Simulated/Actual)	Group	Rate(ai/a)	Treatment Interval	Total # Treatments	Mor.(%)		
Species:	Control						
	Treatment I						
Lab:	Treatment II						
Acc.	Treatment III						
	Crop/Site:		Study Duration:				
	Comments:						

Chronic fish,
Species
Lab:
Acc.

Concentrations Tested (pp__)= _____
MATC = >____ <____ pp__. Effected Parameter = _____
Contr. Mort.(%)= _____ Sol. Contr. Mort.(%)= _____
Comments:

Chronic invertebrate
Species Daphnia magna
57.3%
Lab Analytical Bio-Chemistry Laboratories, Inc.
Acc. 408937-05

Concentrations Tested (ppm)= 0.34, 0.65, 1.2, 2.1, 4.6
MATC => 1.2 < 2.1 ppm. Effected Parameter(s) length and young/adult/reproduction day
Contr. Mort.(%)= 0 % Sol. Contr. Mort.(%)= N/A.
Comments: Based on mean measured concentrations.
K.R. Core
07/06/89

TABLE 3

Percent Survival, Adult Length and Young/Adult/Reproduction Day of Daphnia magna Continuously Exposed to ^{14}C-SC-0224 During a 21-Day Life Cycle Study

Chamber I.D. (nominal concentrations)	Mean Measured Concentration (mg/l)	Mean[a] Percent Survival	Adult[a] Mean Length (mm)	Mean[a] Young/Adult/ Reproduction Day
Control	---	92	4.0	5.5
Level #1 (0.27 mg/l)	0.34	98	3.9	5.4
Level #2 (0.54 mg/l)	0.65	100	4.0	5.4
Level #3 (1.1 mg/l)	1.2	100	3.9	5.2
Level #4 (2.2 mg/l)	2.1	80	3.8[b]	3.4[b]
Level #5 (4.5 mg/l)	4.8	0[b]	0[b]	0[b]

[a]All mean values are calculated directly from the raw data.

[b]Denotes values significantly different ($\alpha \leq 0.05$) from the pooled controls using one-way analysis of variance (ANOVA) and Dunnett's Multiple means test.

Sulfosate
(SC-0224)
Survival

Analysis of Variance File: sulfate Date: 07-04-1994

FILTER: None

N's, means and standard deviations based on dependent variable: SURV
(Arcsine SQRT transformation)

* Indicates statistics are collapsed over this factor

Factors: C Concentration mg/L	N	Mean	S.D.
*	24	1.2122	0.5278
1 Control	4	1.3295	0.1609
2 0.34	4	1.4904	0.1609
3 0.65	4	1.5708	0.0000
4 1.2	4	1.5708	0.0000
5 2.1	4	1.1529	0.3675
* 6 4.8	4	0.1588	0.0000

Fmax for testing homogeneity of between subjects variances: Not defined

Analysis of Variance Dependent variable: SURV

Source	df	SS (H)	MSS	F	P
Between Subjects	23	6.4064			
C (CONC)	5	5.8459	1.1692	37.546	0.0000
Subj w Groups	18	0.5605	0.0311		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.329	6	0.159
2	1.490		
3	1.571		
4	1.571		
5	1.153		

	Comparison	Dunnett
	1 < 2	
	1 < 3	
	1 < 4	
	1 > 5	
*	1 > 6	0.0100
	2 < 3	N.A.
	2 < 4	N.A.
	2 > 5	N.A.
	2 > 6	N.A.
	3 = 4	N.A.
	3 > 5	N.A.
	3 > 6	N.A.
	4 > 5	N.A.
	4 > 6	N.A.
	5 > 6	N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Sulfosate
(SC-0224 Technical)
Reproduction

Analysis of Variance File: sulfate Date: 07-04-1994

FILTER: None

N's, means and standard deviations based on dependent variable: REPROD

* Indicates statistics are collapsed over this factor

Factors: C Concentration mg/L	N	Mean	S.D.
*	24	4.1533	2.0458
1 Control	4	5.5000	0.1831
2 0.34	4	5.3850	0.2462
3 0.65	4	5.3925	0.0532
4 1.2	4	5.2000	0.4389
*5 2.1	4	3.4425	0.4191
*6 4.8	4	0.0000	0.0000

Fmax for testing homogeneity of between subjects variances: Not defined

Analysis of Variance Dependent variable: REPROD

Source	df	SS (H)	MSS	F	P
Between Subjects	23	96.2637			
C (CONC)	5	94.8681	18.9736	244.705	0.0000
Subj w Groups	18	1.3957	0.0775		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	5.500	6	0.000
2	5.385		
3	5.392		
4	5.200		
5	3.443		

Comparison	Dunnett
1 > 2	
1 > 3	
1 > 4	
*1 > 5	0.0100
*1 > 6	0.0100
2 < 3	N.A.
2 > 4	N.A.
2 > 5	N.A.
2 > 6	N.A.
3 > 4	N.A.
3 > 5	N.A.
3 > 6	N.A.
4 > 5	N.A.
4 > 6	N.A.
5 > 6	N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Sulfosate
(SC-0224 Technical)
Length

Analysis of Variance File: SULFDAP Date: 07-04-1994

FILTER: None

N's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

Factors: C	R	Concentration mg/L	N	Mean	S.D.
*	*		187	78.5722	2.6577
1	*	Control	37	79.1622	2.4440
2	*	0.34	39	78.7436	2.4465
3	*	0.65	40	79.2750	2.5012
4	*	1.2	40	78.3000	2.8752
* 5	*	2.1	31	77.0968	2.5866
*	1		43	78.9070	3.0065
*	2		47	77.9149	2.2050
*	3		50	78.9000	2.4764
*	4		47	78.5745	2.8724
1	1		9	77.6667	1.8708
1	2		9	77.0000	1.9365
1	3		10	81.3000	1.6364
1	4		9	80.4444	1.1304
2	1		10	80.2000	2.3476
2	2		10	77.9000	2.0248
2	3		10	78.4000	2.5906
2	4		9	78.4444	2.5055
3	1		10	79.0000	3.0551
3	2		10	79.8000	1.8135
3	3		10	79.1000	2.1833
3	4		10	79.2000	3.0478
4	1		10	79.8000	3.8816
4	2		10	76.9000	2.3310
4	3		10	77.9000	1.9120
4	4		10	78.6000	2.5906
5	1		4	76.0000	1.8257
5	2		8	77.8750	1.8851
5	3		10	77.8000	2.5298
5	4		9	76.1111	3.2575

Fmax for testing homogeneity of between subjects variances: 11.79
Number of variances= 20 df per variance= 8.

Analysis of Variance Dependent variable: LENGTH

Source	df	SS (H)	MSS	F	P
Between Subjects	186	1313.7753			
C (CONC)	4	104.2279	26.0570	4.419	0.0020
R (REP)	3	29.0594	9.6865	1.643	0.1799
CR	12	195.7800	16.3150	2.767	0.0018
Subj w Groups	167	984.7080	5.8965		

Analysis of Variance File: SULFDAP Date: 07-04-1994

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean
1	79.162
2	78.744
3	79.275
4	78.300
5	77.097

	Comparison	Dunnett
	1 > 2	
	1 < 3	
	1 > 4	
*	1 > 5	0.0100
	2 < 3	N.A.
	2 > 4	N.A.
	2 > 5	N.A.
	3 > 4	N.A.
	3 > 5	N.A.
	4 > 5	N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Post-hoc tests for factor R (REP)

Level	Mean
1	78.907
2	77.915
3	78.900
4	78.574

Comparison	Dunnett
1 > 2	
1 > 3	
1 > 4	
2 < 3	N.A.
2 < 4	N.A.
3 > 4	N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).